Myeloperoxidase Gene Variation as a Determinant of Atherosclerosis Progression in the Abdominal and Thoracic Aorta: An Autopsy Study

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SUMMARY: Myeloperoxidase (MPO) is an enzyme that transforms low-density lipoprotein into atherogenic particles. The MPO gene has a promoter polymorphism at position -463, which affects gene transcription and leads to high- (G/G) and low-expression (A/A, A/G) genotypes. To determine if these genotypes are associated with the severity of atherosclerosis, we performed an autopsy study of 300 men aged 33 to 69 years (Helsinki Sudden Death Study). We examined the percentage area of fatty streaks and fibrotic, calcified, and complicated lesions using computer-assisted planimetry. The MPO genotypes were determined by PCR. There were significant interactions of MPO genotype with the mean area of fibrotic ($p < 0.01$) and calcified ($p < 0.05$) lesions in the abdominal aorta and in fibrotic lesions in the thoracic aorta ($p = 0.003$). In the abdominal aorta, men < 53 years with low-expression genotypes had on average a 38.6% larger area of fibrotic lesions and a 43.8% larger area of calcified lesions than did the subjects with the G/G genotype. This association weakened with advancing age. Among men < 53 years, the MPO genotype was an independent predictor of fibrotic ($p = 0.037$) and calcified ($p = 0.001$) lesion area in the abdominal aorta after adjustment for age, body mass index, diabetes, hypertension, and smoking. MPO gene variation may modify the extent of advanced atherosclerotic lesions in the human aorta in early middle age. (*Lab Invest* 2003, 83:919–925).

Atherosclerosis is characterized by an inflammatory component as evidenced by the presence of cells of the immune system, such as phagocytes, while the atherosclerotic lesion develops (Berliner et al, 1995; Ross, 1999). Once activated, the phagocytic cells generate reactive oxygen species that induce destructive oxidizing stress to the vessel wall (Babior, 2000). In atherosclerosis, the chronic inflammation of the artery wall leads to an exaggerated response of the phagocytic cells, which results in oxidation of low-density lipoprotein (LDL). LDL particles become atherogenic. During the continuing inflammation, the activity of the phagocytic cells is thought to lead to an inflammatory response in the vessel wall, which involves cellular excretion and proliferation that results

in further growth of the lesion (Berliner and Heinecke, 1996; Heinecke, 1998, 1999; Rosenfeld, 1998; Steinberg, 1997).

Myeloperoxidase (MPO) is a heme enzyme found in phagocytes and is considered to be an essential part of the antimicrobial defense system (Klebanoff, 1980; Winterbourn et al, 2000). During the oxidative burst related to phagocyte activation, MPO generates a number of reactive species, such as hypochlorous acid (Harrison and Schultz, 1976), tyrosyl radicals (Heinecke et al, 1993), and reactive nitrogen species (Eiserich et al, 1998; Harrison and Schultz, 1976; Hazen et al, 1996). Reactive intermediates generated by MPO of the coronary wall are thought to constitute an important pathway of oxidative destruction throughout the disease process of atherosclerosis (Heinecke, 1999). MPO is expressed in human atherosclerotic lesions, and stable end-products generated by MPO-derived oxidants are enriched in proteins, lipids, and LDL of the lesions (Daugherty et al, 1994; Hazell et al, 1996; Hazen and Heinecke, 1997; Leeuwenburgh et al, 1997; Malle et al, 2000). Furthermore, in patients with coronary artery disease (CAD) leukocyte- and blood-MPO levels are greater than in controls (Zhang et al, 2001). The protective effect of the MPO deficiency against cardiovascular damage has also been reported (Kutter et al, 2000).

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The promoter region of the *MPO* gene has a single G-to-A base substitution at position -463 inside a strong SP1 transcription factor consensus sequence. This polymorphism leads to high- (G/G) and low-expression (A/A, A/G) genotypes in promoter activity and gene expression assays in vitro (Piedrafita et al, 1996). The polymorphism has been previously associated with a susceptibility to several disease states, such as acute promyelocytic leukemia (Reynolds et al, 1997), multiple sclerosis (Nagra et al, 1997), lung cancer (London et al, 1997), and Alzheimer's disease (Reynolds et al, 1999). Whether this genetic variation of the *MPO* gene is associated with atherosclerosis is not clear. In patients with angiographically documented CAD, the A allele is less frequent (Nikpoor et al, 2001). In contrast it is also known that the A allele is associated with increased lipid levels in the healthy population, suggesting a possible implication of this allele with the risk of CAD (Hoy et al, 2001).

This prompted us to investigate whether the *MPO* genotypes modify the extent of different types of atherosclerotic lesions in the thoracic and abdominal aorta. The study was performed on an autopsy series of 300 middle-aged Finnish men from the Helsinki Sudden Death Study with autopsy-confirmed atherosclerosis in the abdominal and thoracic aorta (Ilveskoski et al, 1999).

Results

Study Population

The data from autopsies and of *MPO* genotypes were available in 266 cases, which comprised the final study population. To study the effect of age, the subjects were divided according to median age of 53 years, a cut-off point previously used in the same series (Ilveskoski et al, 1999). The background characteristics and the categorized *MPO* genotype frequencies for both age groups are shown in Table 1. Among the 266 autopsies of men aged 33 to 69 years,

the *MPO* genotype frequencies were 180 (67%) for G/G, 79 (30%) for A/G, and 7 (3%) for A/A. The frequencies of the G and A alleles were 0.81 and 0.19 for men < 53 years and 0.84 and 0.16 for men ≥ 53 years, respectively. There were no significant differences in genotype or allele distributions between these two age groups. The distributions of the genotypes followed the Hardy-Weinberg equilibrium. There were no significant differences between genotype or age groups and the characteristics of the study subjects, with the exception of the consumption of alcohol, which was significantly higher among men < 53 years compared with older men.

The number of cases without atherosclerotic lesions and the proportion of the subjects affected by the atherosclerotic disease are presented in Table 2. For data on the various types of atherosclerotic lesions, see Table 3.

MPO Gene Variation and Atherosclerotic Lesions in Abdominal Aorta

In analysis of covariance (ANCOVA), age and body mass index (BMI) were used as covariates and the *MPO* genotype and age group as factors. There were significant *MPO* genotype-by-age interactions for the percent area of both fibrotic ($p = 0.008$) and calcified ($p = 0.015$) atherosclerotic lesions in the abdominal aorta. Men < 53 years with the low-expression genotypes (A/G, A/A) had, on average, a 38.6% larger area of fibrotic lesions ($p = 0.017$) and a 43.8% larger area of calcified lesions ($p = 0.026$) than did the subjects with the high-expression genotype (G/G) (Table 3). The power of the test measuring the differences between the areas of calcified and fibrotic lesions by *MPO* genotype was 99% for fibrotic and 91% for calcified lesions (124 cases in each group). By linear regression analysis, which included all available atherosclerosis risk factors, *MPO* genotype turned out to be an independent predictor of fibrotic ($p = 0.04$) and calci-

Table 1. Subjects by *MPO* Genotype and Age

<i>MPO</i> genotype group	< 53 Years old		≥ 53 Years old		All subjects	
	AA/AG	GG	AA/AG	GG	AA/AG	GG
No. of subjects	42	83	44	97	86	180
Age, years ^a	42.93 ± 4.20	43.17 ± 4.44	60.32 ± 5.18	59.92 ± 4.75	51.83	52.19
Body mass index, kg/m ^{2a}	24.80 ± 5.18	24.49 ± 4.66	24.30 ± 4.23	25.97 ± 5.18	24.55	25.29
Myocardial infarction (yes/no)	8/34 (19%)	9/74 (11%)	15/29 (34%)	34/63 (35%)	23/63 (27%)	43/137 (24%)
Coronary thrombus (yes/no)	2/40 (5%)	5/78 (6%)	4/40 (9%)	9/88 (9%)	6/80 (7%)	14/166 (8%)
Cause of death, <i>N</i>						
Cardiac disease/other ^b	14/28 (33%)	21/62 (25%)	24/20 (55%)	46/51 (47%)	38/48 (44%)	67/113 (37%)
<i>MPO</i> genotypes, <i>N</i> (%)	42 (16%)	83 (31%)	44 (17%)	97 (36%)	86 (32%)	180 (68%)
Coronary risk factor						
Diabetes (yes/no) ^c	1/16 (6%)	5/37 (12%)	4/20 (17%)	9/34 (21%)	5/36 (12%)	14/71 (16%)
Hypertension (yes/no) ^c	4/13 (24%)	12/30 (29%)	8/16 (33%)	15/28 (35%)	12/29 (29%)	27/58 (32%)
Smoking (yes/no) ^d	13/9 (59%)	34/7 (83%)	18/12 (60%)	34/16 (68%)	31/21 (60%)	68/23 (75%)
Alcohol g/d ^{ac}	158.3 ± 121.0	118.5 ± 101.5	96.7 ± 124.4	70.3 ± 82.5	124.8 ± 125.4	92.2 ± 94.2

MPO, myeloperoxidase.

^a Values are mean ± standard deviation.

^b Includes causes of death due to other diseases, violence, intoxication, and unknown reasons (*N* = 6).

^c Data available in 126 cases.

^d Data available in 143 cases; ex-smokers are included in the group of nonsmokers.

Table 2. The Number and Proportion of Cases with or Without Different Atherosclerotic Lesion Types in Abdominal and Thoracic Aorta

	< 53 Years old (<i>N</i> = 125)			≥ 53 Years old (<i>N</i> = 141)			All (<i>N</i> = 266)		
	Lesions		Affected, %	Lesions		Affected, %	Lesions		Affected, %
	No	Yes		No	Yes		No	Yes	
Abdominal aorta									
Fatty streak	0	125	100	0	141	100	0	266	100
Fibrotic	20	105	84	1	140	99.3	21	239	89.8
Calcified	42	83	66.4	3	138	97.9	45	221	83.1
Thoracic aorta									
Fatty streak	8	117	93.6	1	140	99.3	9	257	96.6
Fibrotic	60	65	52	11	130	92.2	71	195	73.3
Calcified	96	29	23.2	27	114	80.9	123	143	53.8

fied ($p = 0.001$) lesion area in men < 53 years (Table 4). No statistically significant associations for MPO genotype were found among men ≥ 53 years. For the other types of lesions in abdominal aorta, there were no significant differences between the study groups (Table 3).

In ANCOVA involving all of the known risk factors as covariates, the interaction remained significant in fibrotic ($p = 0.038$) and in calcified ($p = 0.053$) lesion areas in abdominal aorta. When the possible confounding factor, alcohol consumption, was added to the linear regression analysis as an independent variable, MPO genotype remained as an explanatory variable in fibrotic ($p = 0.055$) and calcified ($p = 0.001$) lesions in abdominal aorta among men < 53 years; however, the entire regression model was not significant in fibrotic lesions.

MPO Gene Variation and Atherosclerotic Lesions in Thoracic Aorta

By ANCOVA, there was a statistically significant ($p = 0.003$) interaction between MPO genotype and age group regarding the fibrotic lesion area in the thoracic aorta when age and BMI were used as covariates. Men ≥ 53 years with high-expression genotype (G/G) had on average a 24.5% larger area of fibrotic lesions ($p = 0.012$) than subjects with low-expression genotypes (A/G, A/A) (Table 3). The power of the test to measure the difference between the area of fibrotic lesions according to MPO genotypes was 80% with 136 cases. In addition, MPO genotype did not remain as an independent predictor of fibrotic lesions when analyzed by linear regression adjusted for the available atherosclerosis risk factors. There were no associations found among men < 53 years, and there were no significant differences between the age-genotype groups as the other types of lesions were concerned (Table 3).

For the statistically significant areas of atherosclerotic lesions in abdominal and thoracic aortas, the one-way ANOVA (ANOVA) was performed broken down by age quartiles and using the MPO genotype as a factor (Table 5).

Discussion

To our knowledge, no previous studies have investigated the association between MPO gene promoter polymorphism and the severity of atherosclerosis using arterial wall samples obtained at autopsies. We found an age-dependent association between MPO gene promoter −463G/A genotype and the percent area of fibrotic and calcified atherosclerotic lesions in the aorta. The MPO low-expression genotypes (A/A and A/G) were associated with larger fibrotic and calcified lesions in men < 53 years in both ANCOVA and linear regression analysis, but the effect was abrogated as the age exceeded 53 years. Men ≥ 53 years carrying the MPO high-expression genotype (G/G) had larger areas of fibrotic lesions, especially in the thoracic aorta and to a lesser degree also in the abdominal aorta. However, this association is suggestive, and the MPO genotype did not appear as an independent predictor in linear regression analysis among men ≥ 53 years.

The proportions of the MPO genotypes in our study population were comparable to what has previously been published concerning the general Caucasian population (Reynolds et al, 1997). In earlier reports MPO −463G/A polymorphism has been studied in several disease states. The A allele has been reported to have a protective role in the occurrence of symptomatic and angiographically proved CAD, but the stage of possible asymptomatic CAD of the controls was not evaluated in the study (Nikpoor et al, 2001). The G/G genotype is claimed to be a risk factor in lung cancer (London et al, 1997), but in Finnish men no direct association has been found (Misra et al, 2001). In addition, no difference in serum MPO levels between the genotypes has been detected (Hoy et al, 2001). Our results imply that the high-expression genotype G/G offers protection from the development of atherosclerosis. In line with our observation, a recent report found that subjects carrying the A allele displayed higher levels of atherosclerosis risk factors, triglycerides, total cholesterol, LDL cholesterol, and apolipoprotein B than subjects with the G allele. (Hoy et al, 2001). Owing to the sudden and unexpected

Table 3. Mean Percent Area of Different Atherosclerotic Lesion Types in Abdominal and Thoracic Aortas by MPO Genotype and Age^a

	< 53 Years old				≥ 53 Years old				<i>p</i> , ANCOVA ^b		
	AA/AG (N = 41)	GG (N = 83)	All (N = 124)	AA/AG (N = 42)	GG (N = 94)	All (N = 136)	MPO	Age group	Interaction		
Abdominal aorta											
Fatty streak	11.87 ± 11.00	13.27 ± 10.09	12.38 ± 10.37	13.23 ± 11.49	14.96 ± 10.63	14.42 ± 10.89	0.125	0.735	0.750		
Fibrotic	6.17 ± 5.84	3.79 ± 4.28 ^c	4.56 ± 4.94	6.53 ± 5.41	7.19 ± 5.07	6.98 ± 5.17	0.359	0.035	0.008		
Calcified	1.63 ± 2.06	0.90 ± 2.00 ^c	1.14 ± 2.04	5.61 ± 8.62	5.26 ± 5.36	5.37 ± 6.51	0.344	0.454	0.015		
Thoracic aorta											
Fatty streak	7.01 ± 10.63	6.92 ± 6.03	6.95 ± 7.81	7.74 ± 6.75	10.36 ± 6.90	9.55 ± 6.93	0.060	0.999	0.214		
Fibrotic	2.69 ± 5.11	1.67 ± 3.20	2.00 ± 3.94	5.10 ± 6.13	6.84 ± 5.89 ^d	6.31 ± 6.00	0.500	0.615	0.003		
Calcified	0.20 ± 0.46	0.06 ± 0.18	0.11 ± 0.31	1.95 ± 3.05	1.52 ± 3.10	1.65 ± 3.08	0.342	0.476	0.528		

ANCOVA, analysis of covariance; MPO, myeloperoxidase.

^aData analysis was based on two-way ANCOVA wherein MPO genotype and age subgroup were used as factors and age and body mass index were used as covariates.^b*p* values derived from two-way ANCOVA after square-root formation; MPO and age groups are the main effects for the two-way interaction.^cValues are mean ± standard deviation.^d*p* < 0.05 for the difference between genotypes; least significant difference test was used as a post hoc test.

nature of death, the possible confounding effect of serum cholesterol or other lipid levels in the deceased were not available and could not been taken into account in statistical analysis.

The effect of the MPO polymorphism on atherosclerosis development was age dependent, and the interaction was stronger in men < 53 years. This finding is somewhat in agreement with the conclusions of the Swedish twin study (Marenberg et al, 1994), wherein genetic factors were suggested to play a greater role in death due to coronary heart disease at a younger age, and at an older age, the effect of environmental or acquired factors tended to dampen the inherited dispositions. It is also possible that our results are due to linkage disequilibrium of the -463G/A polymorphism, with some other functional MPO polymorphism being responsible for more advanced atherosclerosis.

There were significant interactions of MPO genotype with lesions with fibrous tissue and calcification. According to the inflammation theory of atherosclerosis development, the presence of phagocytic cells in the developing atheroma is thought to contribute to the continuing accumulation of oxidized LDL; this leads to increased matrix synthesis in fibrotic lesions and then to a calcification process (Berliner et al, 1995; Berliner and Heinecke, 1996; Lusis, 2000; Ross, 1999). MPO and proteins modified by MPO have been detected from advanced human atherosclerotic plaques, whereas only a small amount of MPO is present in human coronary artery fatty streaks (Daugherty et al, 1994; Heinecke, 1998; Malle et al, 2000; Sugiyama et al, 2001). This is congruent with our study, in which no significant differences were found between the genotypes in the mean area of fatty streaks.

In summary, the results of our study suggest that there is an interaction for the genetic variant of the *MPO* gene with the progression of atherosclerosis, similar to that previously found for apolipoprotein E epsilon 4 (Ilveskoski et al, 1999). The precise role of MPO and its interaction with other genetic and acquired factors involved in the development of atherosclerosis require more investigation.

Materials and Methods

Subjects

The Helsinki Sudden Death Study was designed to study the acquired and inherited risk factors predisposing to sudden death in Finnish middle-aged men. An autopsy series including 300 consecutive Caucasian men aged 33 to 69 years was collected during 12 months from 1991 to 1992 at the Department of Forensic Medicine, University of Helsinki, as previously described (Ilveskoski et al, 1999; Mikkelsen et al, 2000). The cause of death was cardiac disease in 39% (*n* = 105), other diseases in 21% (*n* = 55), and violent death (suicides and accidents) in 40% (*n* = 106) (Table 1).

Table 4. Strongest Predictors of the Area of Fibrotic and Calcified Atherosclerotic Lesions in Abdominal Aorta^a

Age group	Explanatory variables (independent)	Fibrotic lesions		Calcified lesions	
		Beta ^b	p Value	Beta ^b	p Value
< 53 Years	MPO genotype	0.265	0.037	0.398	0.001
	BMI	−0.021	0.877	−0.110	0.347
	Age	0.320	0.015	0.456	0.000
	Hypertension (yes or no)	0.213	0.102	0.069	0.539
	Diabetes (yes or no)	0.095	0.454	0.185	0.100
	Entire model (R ²)	0.231	0.017	0.324	< 0.001
≥ 53 Years	MPO genotype	−0.118	0.378	−0.011	0.933
	BMI	−0.033	0.817	−0.313	0.023
	Age	0.182	0.172	0.185	0.143
	Hypertension (yes or no)	−0.067	0.632	0.109	0.414
	Diabetes (yes or no)	−0.055	0.671	−0.016	0.897
	Entire model (R ²)	0.053	0.667	0.054	0.146

BMI, body mass index; MPO, myeloperoxidase.

^a Linear regression analysis was carried out in both age groups to identify the independent risk factors for atherosclerotic lesions.

^b Standardized coefficients.

Measuring the Area of Atherosclerotic Lesions by Computer-Assisted Morphometry

The definition of atherosclerosis was based on the protocols of two international studies: the International Atherosclerosis Project, Standard Operating Protocol (Guzman et al, 1968); and the WHO Study Group in Europe (Uemura et al, 1964). Whole-mounted proximal parts of the abdominal and thoracic aorta were collected for analysis. The area involved with fatty streaks, fibrotic plaques, and calcified lesions was measured by computer-assisted planimetry, which is a standard planimetric machine connected to a personal computer. The procedure of the atherosclerotic lesion measurements has been described by Ilveskoski et al (1999).

Risk Factors for Atherosclerosis Disease

A spouse, relative, or close friend of the deceased was reached and interviewed in 147 cases. Among the questions pertaining to the risk factors for sudden death (ie, arterial hypertension and diabetes), there were questions to establish past and recent smoking and drinking habits of the deceased. Complete data on all risk factors in addition to MPO genotype and autopsy data were available in 126 cases, which comprised the adjusted study population.

Determination of the MPO Genotype

DNA was isolated from frozen cardiac muscle samples by a standard phenol extraction method. A region surrounding the −463G/A variant of the *MPO* gene was amplified using PCR, and the primary and secondary round primers were designed as previously described by London and colleagues (London et al, 1997). After digestion of the PCR product with *Acil* restriction endonuclease (New England Biolabs Inc., Beverly, Massachusetts), fragments were separated

using agarose gel (2%) electrophoresis and were visualized by ethidium bromide staining.

Statistical Analyses

Data analysis was based on two-way ANCOVA and linear regression. Non-normally distributed data were square-root transformed prior to analysis, but the results are displayed as the crude values. To achieve a suitable group size, MPO genotypes were categorized into high-expression (G/G) and low-expression (A/G, A/A) allele groups, a categorization previously used in another study (Reynolds et al, 2000). To adjust for possible differences between younger persons whose atherosclerosis process was not far advanced and older persons with a longer disease period, the data were divided into two age groups, < 53 and ≥ 53 years old by the median age (53 years). The MPO genotype and age subgroups were used as factors in the two-way ANCOVA, in which the possible confounding effects of age and BMI were taken into account by including them into the model as covariates. The least significant difference test was used as a post hoc test to study the differences between the genotype groups. We also used linear regression analysis to identify independent risk factors for atherosclerotic lesions in both age groups. The analyses were carried out separately in both age subgroups. The mean areas of fibrotic and calcified lesions were included as independent variables in two separate models in which MPO genotype group, age, BMI, hypertension (yes/no), diabetes (yes/no), and smoking (yes/no) were included as explanatory variables. One-way ANOVA was used for the statistical analysis broken down by the age quartiles, and MPO genotype was used as a factor for the analysis. The statistical analyses were performed using Statistica for Windows 5.1 (StatSoft Inc., Tulsa, Oklahoma), and SPSS 9.0 for Windows 95 software (SPSS Inc., Chicago, Illinois). The power (1-β) of the test procedures was calculated

Table 5. Mean Percent Area of Fibrotic and Calcified Atherosclerotic Lesion Types Broken Down by Age Quartiles and MPO Genotype Group

	33–44 Years		> 44–53 Years		> 53–60 Years		> 60–69 Years	
	AA/AG N = 23	GG N = 49	AA/AG N = 23	GG N = 43	AA/AG N = 18	GG N = 41	AA/AG N = 19	GG N = 44
Abdominal aorta ^a								
Fibrotic	5.09 ± 5.60	3.13 ± 3.80	6.84 ± 5.60	4.68 ± 4.40 ^b	6.58 ± 6.69	6.31 ± 4.76	6.95 ± 4.55	8.59 ± 5.40
Calcified	1.48 ± 2.49	0.38 ± 0.80 ^c	1.76 ± 1.30	2.16 ± 3.16	3.97 ± 6.48	4.52 ± 5.52	8.19 ± 10.75	6.21 ± 5.42
Thoracic aorta ^a								
Fibrotic	1.40 ± 2.61	1.39 ± 3.55	3.81 ± 6.34	2.17 ± 2.63	5.83 ± 8.31	7.12 ± 5.93 ^b	5.36 ± 4.00	7.46 ± 6.04

MPO, myeloperoxidase.

^aValues are mean ± standard deviation.^b*p* < 0.10 for the difference between genotypes; *p* values are derived from one-way analysis of variance after square-root formation using the MPO group as a factor.^c*p* < 0.05 for the difference between genotypes.

by using the Power and Sample Size (PS) program version 1.0.15 for Windows 95. A test with a power of 80% was considered to be statistically reliable.

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